Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta 1608 (2004) 149–154

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.bba-direct.com

Different sensitivities of CPT I and CPT II for inhibition by L-aminocarnitine in human skeletal muscle

Kathrin Traufeller, Frank Norbert Gellerich*, Stephan Zierz

Muskellabor der Neurologischen Klinik und Poliklinik, der Martin-Luther Universität Halle-Wittenberg, Julius-Kühn-Strasse 7, D-06097 Halle an der Saale, Germany

Received 6 May 2003; received in revised form 18 November 2003; accepted 18 November 2003

Abstract

L-Aminocarnitine (L-AC) has been shown to inhibit carnitine palmitoyltransferases (CPT) in rat muscle and in rat liver. However, there are no reports on interactions of L-AC with CPT II and CPT I of human muscle. Therefore, the aim of the present work was to characterize the inhibition of human muscle CPT I and CPT II by L-AC in muscle mitochondria, skinned fibers and muscle homogenates in comparison to the established action of malonyl-CoA. Both isoenzymes were inhibited by L-AC, but sensitivity was different (CPT I, K_d = 3.8 mM L-AC; CPT II, K_d = 21.3 μM L-AC). A mixed inhibition type in respect to carnitine was detected (K_i = 3.5 μM L-AC). At 0.5 mM L-AC, CPT II was completely inhibited without affection of CPT I. In contrast, CPT I was completely inhibited by 0.4 mM malonyl-CoA (K_d = 0.5 μM), whereas CPT II was nearly not affected by this inhibitor. Using these inhibitors in muscle homogenates, activities of CPT II and CPT I were detected to be 38 ± 10% and 63 ± 10% of total, respectively (n = 21). In intact mitochondria and different fractions of muscle homogenates after selective solubilization of CPT II by Tween 20, the extent of specific CPT inhibition changed in relation to the accessible isoenzyme pattern. Palmitoyl-carnitine-dependent respiration in skinned fibers was inhibited by high concentrations of L-AC, indicating that the inhibitor can be transported via the acyl-carnitine transporter, too. The combined use of both inhibitors (L-AC and malonyl-CoA) allows the kinetic characterization of CPT I and CPT II in human muscle homogenates. In addition, it has been shown that L-AC can be used for the study of metabolic consequences of CPT II deficiency on function of intact mitochondria.

Keywords: CPT; Human muscle mitochondria; L-aminocarnitine; Inhibition

1. Introduction

Carnitine palmitoyltransferases (CPT I and CPT II) together with the acyl-carnitine translocator mediate the transfer of acyl-groups into mitochondria. For review see Ref. [1]. Various diseases, due to defects in enzymes of acyl-carnitine transport, are known [2–5]. The most frequently affected enzyme is CPT II [6], clinically almost entirely restricted to skeletal muscle and characterized by recurrent attacks of myoglobinuria [2,5,6]. More than 25 pathogenic mutations have been detected in the human CPT II gene [7–11] associated with abnormal regulatory properties of the enzyme. Detailed kinetic consequences, however, on the phenotype of the enzyme are unknown. Such investigations could be done in biopsies or cell cultures taken for diagnostic purpose.

For kinetic investigations of CPT II in muscle homogenates, CPT I can be specifically inhibited by etomoxir [12] or the physiological metabolite malonyl-CoA [1]. L-AC is an inhibitor of CPT [13–16]. It can be converted into acetyl-L-AC which cannot be metabolized further [16]. There was the idea to use L-AC as antiketogenic and hypoglycaemic drug [17]. For that purpose, in vitro and in vivo studies in rats were performed to characterize the effect of L-AC on CPT. It has been shown that L-AC inhibits the activity of CPT in homogenates of rat liver mitochondria by 64% [17]. At first, it was assumed that L-AC inhibits both CPT isoenzymes in preparations of rat liver mitochondria [17]. Subsequent experiments on rat hearts suggested that L-AC should be a specific inhibitor of CPT II [15]. This result was confirmed on rat liver

Abbreviations: BCA, bicinchoninic acid assay; CPT, carnitine palmitoyltransferase; L-AC, L-aminocarnitine; NCP, non-collagen protein

* Corresponding author. Tel.: +49-345-557-3628; fax: +49-345-557-3505.

E-mail address: frank.gellerich@medizin.uni-halle.de (F.N. Gellerich).

0005-2728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2003.11.005

mitochondria where 0.8 μM L-AC inhibited CPT II but not CPT I [14]. However, there are no reports on effects of L-AC on CPT II and CPT I of human muscle.

The aim of the present study was to investigate the action of L-AC on CPT I and CPT II in homogenates of human skeletal muscle, in isolated mitochondria, and in skinned muscle fibers. Results demonstrate the possibility to investigate kinetically CPT I as well as CPT II in muscle homogenates of human muscle biopsies using specific inhibitors (L-AC and malonyl-CoA) without the necessity to separate them from each other.

2. Materials and methods

Muscle specimens were obtained from patients who had muscle biopsy for diagnostic purposes but were ultimatively found to have no neuromuscular disease by combined clinical, electrophysiological, histological, and biochemical criteria. Muscle specimens were taken from m. quadriceps (m. vastus lateralis) or m. biceps brachii by open biopsy under local anesthesia. Samples were immediately frozen and stored in liquid nitrogen until subsequent biochemical studies. For investigation of mitochondrial function from some patients, a part of muscle biopsy was stored on ice at 4 °C in high-energy preservation solution [18] after rough dissection. It has been shown that mitochondrial function remains intact up to 30 h under these conditions [19]. Written informed consensus was obtained from all patients before biopsy.

2.1. Isolation and permeabilization of muscle fibers

Immediately before oxygraphic measurements, the fibers were permeabilized for 30 min with 50 μg saponin/ml of preservation solution consisting of 8.1 mM K-EGTA, 1.9 mM CaEGTA buffer (free Ca^{2+} concentration 0.1 μM), 9.5 mM $MgCl_2.6H_2O$, 3 mM KH_2PO_4, 20 mM taurine, 5.2 mM ATP, 15 mM PCr, 49 mM K^+-MES, 20 mM imidazole (pH 7.1) as described previously [18]. After permeabilization, the fibers were washed three times (10 min) in incubation medium (see below) to remove saponin and adenine nucleotides. All procedures were performed in a cooling-room on ice at 4 °C.

2.2. Respirometric measurements

We used the OROBOROS® oxygraph [20], a two-chamber respirometer with a Peltier thermostat and integrated electromagnetic stirrers. Bundles of fibers (5–10 mg) were transferred into the oxygraph chambers. The measurements were performed at 30 °C in 1.42 ml incubation medium consisting of 75 mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM KH_2PO_4, 0.5 mM EDTA, 5 mM $MgCl_2$, 20 mM Tris–HCl, and 1 mg/ml BSA (pH 7.4) [18]. The oxygen concentration in the air-saturated medium was considered to be 200 nmol O_2/ml at 95 kPa barometric pressure. The weight-specific oxygen consumption was calculated as the time derivative of the oxygen concentration (DATGRAPH Analysis software, OROBOROS®).

2.3. Muscle homogenates

Frozen muscle tissue was homogenized in a glass–glass homogenizer in nine volumes Chappel–Perry buffer containing 50 mM Tris buffer (pH 7.5), 100 mM KCl, 5 mM $MgCl_2$, and 1 mM EDTA [21–23]. For Tween 20 separation of both CPT, 1% Tween 20 was added to the homogenate. After 15 min of incubation, the homogenates were centrifuged for 30 min at 50,000 RPM using a Beckman ultracentrifuge (L7–65) equipped with the rotor 70.1Ti. CPT activity was investigated in the supernatant and in the pellet redissolved with the same concentration of Tween 20.

2.4. Chemicals

All chemicals were obtained from Sigma Chemie (Deisenhofen/Germany). L-AC was a gift from Sigma Tau (Rome/Italy). ^{14}C-carnitine was obtained from NEN Life Science Products (Köln/Germany). All other chemicals were of reagent grade purity.

2.5. Enzyme assays

CPT was determined using a radiochemical isotope forward assay as described previously [21–23]. In brief, the assay system contained 10 mM Tris–HCl buffer (pH 7.6), 0.1% fatty acid-free bovine serum albumin, 1 mM dithiothreitol, 0.08 mM palmitoyl-CoA, and 5 mM L-^{14}C-carnitine in a final volume of 1 ml. Temperature was 30 °C. Experiments were performed either in the presence or in the absence of 0.4 mM malonyl-CoA to inhibit CPT I. Concentration of L-AC was varied between 0 and 30 mM. Incubations were started by the addition of homogenate and stopped after 10 min by the addition of ammonium sulfate and isobutene. Labelled palmitoyl-carnitine was measured after separation of the organic phase using a scintillation counter (Beckmann LS 6500). All measurements were performed in duplicate.

2.6. Protein determination

Non-collagen protein (NCP) was determined by the bicinchoninic acid assay (BCA) [24] (Pierce, Putbus, The Netherlands) after digestion of the homogenate with sodium hydroxide (50 mM) and pelleting the insoluble collagen protein by centrifugation (12,000 × g for 10 min). The BCA reagent was supplemented with 0.1% (w/v) sodium dodecylsulfate. BSA was used as a standard.

3. Results and discussion

3.1. Characterization of CPT inhibition by L-AC and malonyl-CoA

Fig. 1 illustrates the inhibition of CPT I and II in muscle homogenates by L-AC. CPT activity in the absence and in the presence of 0.4 mM malonyl-CoA was measured at increasing concentration of L-AC. Normalized data of three different experiments are shown as means ± S.E. for the total activity (circles). The total CPT activity was inhibited by malonyl-CoA (−59%). Consisting of the prevailing view, the remaining activity was assumed to be CPT II (triangles). The activity of CPT I (squares) was calculated by subtraction of CPT II from the total activity. Increasing additions of L-AC decreased the CPT activity within a large range of concentration. CPT II activity was completely inhibited at 500 μM L-AC, whereas CPT I started to be inhibited at higher concentrations only. We used a general curve fitting-programme which was made for calculation of dissociation constants and further parameters from inhibitor titration curves (see Eq. (5) in Ref. [25]). We found dissociation constants of 21.3 μM and 3.78 mM L-AC for CPT II and CPT I, respectively. Since the inhibitor curve of total CPT was the sum of two curves, it was not possible to obtain a suitable curve fit for CPT I+II.

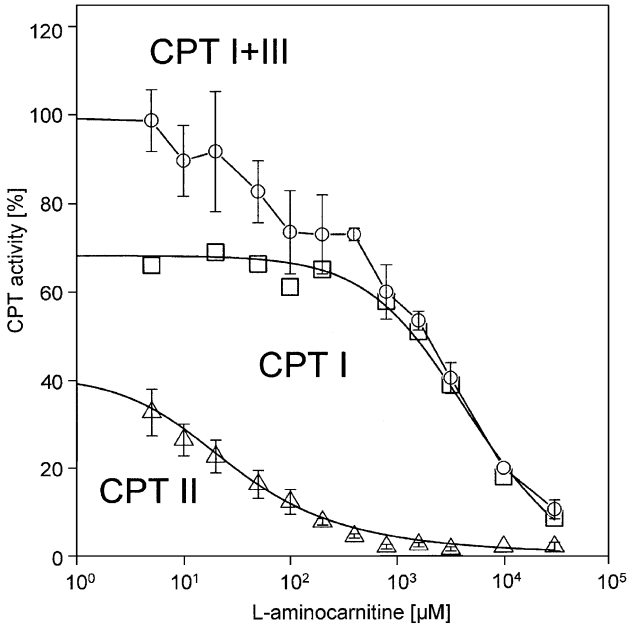

Fig. 1. Inhibition of CPT by L-aminocarnitine. Activity of CPT I+II (circles) was measured radiochemically as described in Section 2.5 using palmitoyl-CoA and L-^{14}C-carnitine (forward assay). Malonyl-CoA insensitive CPT activity (CPT II, triangles) was assayed in the additional presence of 0.4 mM malonyl-CoA. Malonyl-CoA sensitive CPT activity (CPT I, squares) was calculated by subtracting CPT II from the total activity. L-Aminocarnitine was varied between 0 and 30 mM as indicated. Data as means of three typical experiments ± S.E. Activities were expressed as the percentage of the activity seen without the addition of L-AC and malonyl-CoA. Curves were fitted using a nonlinear computer model [25], allowing the estimation of K_d and V_{max}. CPT II: K_d = 21 μM L-AC, V_{max} 41.1%. CPT I: K_d = 3780 μM, L-AC, V_{max} = 68.3%.

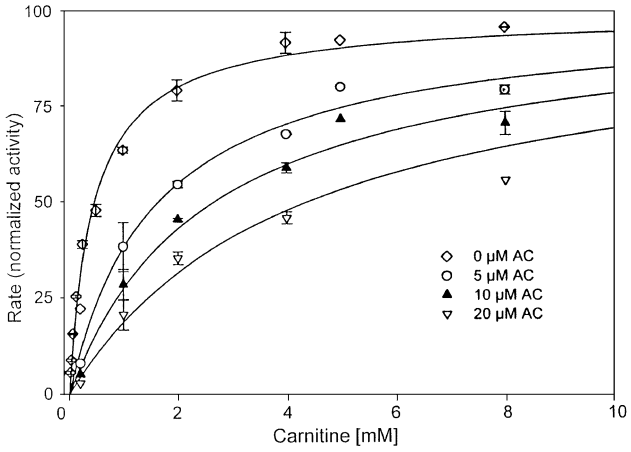

Fig. 2. Inhibition of CPT II by L-aminocarnitine at different concentrations of carnitine. Kinetic investigation of CPT II in muscle homogenates in the presence of 0.4 mM malonyl-CoA. Measurements were performed at varied concentrations of carnitine and L-AC. K_m = 0.46 ± 0.04 mM carnitine, K_i = 1.8 ± 0.3 μM L-AC. Data of three experiments were used for calculation of kinetic constants by means of the kinetic module from Sigma Plot assuming different inhibition types.

Additionally, inhibitor experiments were performed varying the concentration of L-AC and of carnitine as well (Fig. 2). With increasing inhibitor concentration, CPT II activity was increasingly diminished, indicating that a competition occurred between carnitine and L-AC. Using an nonlinear regression programme kinetic constants were estimated to be K_m = 0.46 ± 0.04 mM carnitine and K_i = 1.8 ± 0.3 μM L-AC. The best fit was obtained for a partial competitive inhibition type.

In further experiments, the effect of malonyl-CoA on CPT I and II was studied. In Fig. 3, both CPT activities were plotted versus the inhibitor concentration. CPT I activity decreased sigmoidal with increasing malonyl-CoA concentration (K_d = 0.5 μM), whereas the CPT II activity was only marginally influenced.

3.2. Detection of CPT I/CPT II ratios in human muscle mitochondria and muscle homogenates

L-AC and malonyl-CoA were applied for individual measurements of CPT I and CPT II in muscle homogenates of 21 patients without CPT II deficiency. The relative contents were found to be 38.1 ± 9.8% for CPT II and 63.4 ± 9.5% for CPT I. The sum of both (101.5 ± 7.7%) indicates the good reproducibility of the method. The total activity of CPT I plus CPT II was 1.7 ± 0.5 U/g NCP (n = 21) in m. vastus lateralis of patients without CPT II deficiency.

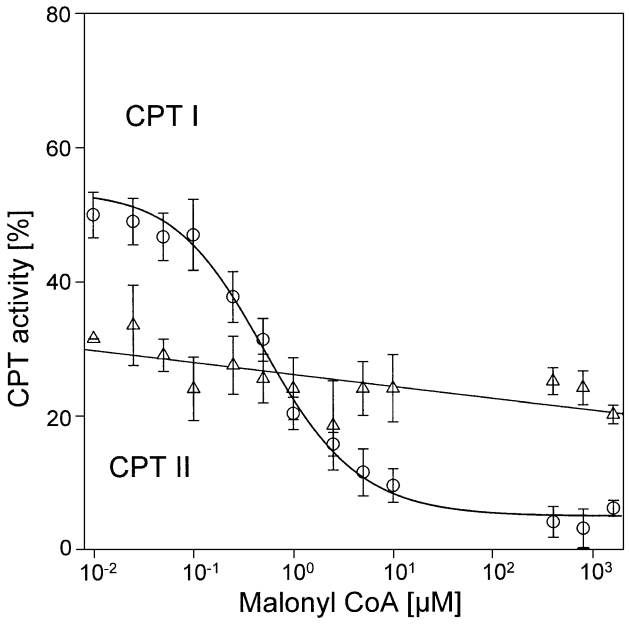

Fig. 3. Inhibition of CPT by malonyl-CoA. Activity of CPT I was measured in homogenates of human skeletal muscle in the presence (circles) of 0.5 mM L-aminocarnitine. CPT II activity (triangles) was calculated subtracting CPT I from the total activity. Means of three typical experiments ± S.E. Activities are expressed as the percentage of the activity measured in the absence of inhibitors.

In further experiments, muscle homogenates were incubated with 1% Tween 20, which is known to selectively release CPT II from mitochondrial inner membranes [22,26]. After ultracentrifugation, both supernatant and pellet fraction were incubated either with 0.5 mM L-AC, with 0.4 mM malonyl-CoA, or without inhibitor. To these incubations, the second inhibitor was added in increasing concentrations as indicated. As shown in Fig. 4A, about 88% of the total CPT activity in the pellet fraction was inhibited by malonyl-CoA (CPT I). The remaining activity was inhibitable by L-AC, indicating that the pellet fraction contained 12% CPT II. Corresponding results were obtained if CPT II was at first inhibited by 500 µM L-AC followed by an inhibitor titration with malonyl-CoA (Fig. 4C). A completely different situation was found in the supernatant. As shown in Fig. 4B, only a very small part of total CPT activity was inhibited by malonyl-CoA, but 80% of CPT was inhibited by 500 µM L-AC (Fig. 4D), indicating that the Tween 20 supernatant indeed contained mainly CPT II.

In isolated functionally intact human muscle mitochondria, accessible CPT activity is CPT I which is localized in the outer membrane. Therefore, as shown in Fig. 5, measurable CPT activity was nearly completely inhibited by 0.2 mM malonyl-CoA but was much less inhibited by 2 mM L-AC. At increasing L-AC concentration (20 mM), CPT I also became inhibited. The lowest activity was measured in the presence of both inhibitors. In disintegrated mitochondria, the matrix enzyme CPT II is accessible, therefore the malonyl-CoA-insensitive enzyme activity was 37.8% similar as described above for muscle homogenates. With increasing L-AC, the remaining activity decreased as expected.

3.3. Inhibition of palmitoyl-carnitine-dependent respiration by L-AC

Fig. 6 shows a respirometric experiment for detection of functional activity of CPT II in intact mitochondria of skinned muscle fibers. Respiration of mitochondria was stimulated by 5 mM ADP in the presence of 2 mM malate only. Respiration under these conditions is low due to the lacking exogenous donor for acetyl-CoA. After addition of 60 µM palmitoyl-carnitine, state 3 respiration

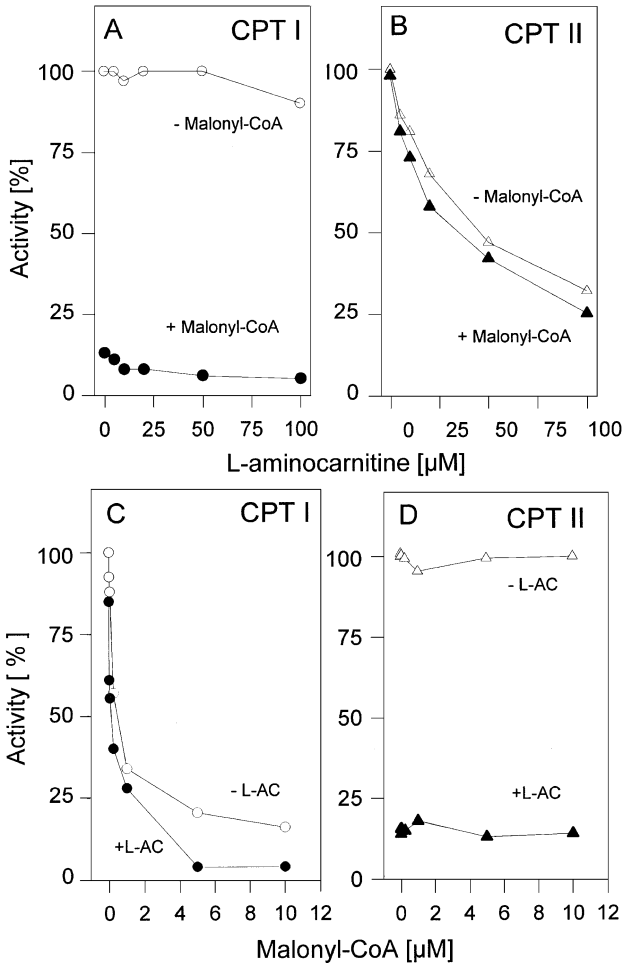

Fig. 4. Inhibition of CPT I and CPT II by malonyl-CoA and L-aminocarnitine in soluble and particulate fractions of human skeletal muscle obtained by Tween 20 fractionation. CPT II was released from muscle mitochondria by incubation of muscle homogenate with 0.5% Tween 20 and separated by centrifugation (soluble CPT II, triangles) from particulate CPT I (pellet, circles). (A,B) Activity of CPT was measured radiochemically without (open symbols) and with (filled symbols) 0.4 mM malonyl-CoA as well as in the presence of L-AC as indicated. (C,D) Activity of CPT was measured without (open symbols) and with (filled symbols) 0.5 mM L-AC. Addition of malonyl-CoA as indicated.

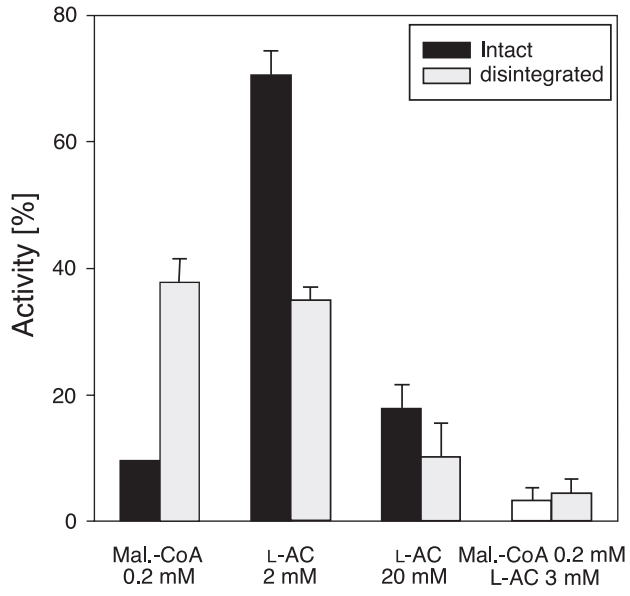

Fig. 5. Different sensitivities of mitochondrial CPT in intact and frozen human muscle mitochondria for malonyl-CoA and L-aminocarnitine. CPT activity was measured in isolated intact human muscle mitochondria and in a mitochondrial homogenate obtained by freezing and thawing. Remaining activity in the presence of indicated inhibitors as percentage of activity measured in the absence of inhibitors. Means of three experiments with double determinations ± S.E.

was adjusted (0.37 nmol O_2/min/mg). Malonyl-CoA had no effect on respiration, but L-AC strongly inhibited the respiratory rate indicating either that the inhibitor was transported into the matrix space under these conditions or that the acyl-carnitine translocator was directly inhibited by L-AC. That the decreased respiration is not a result of unspecific damage of mitochondria by L-AC can be demonstrated by addition of rotenone (inhibitor of complex I) and the subsequent stimulation of respiration

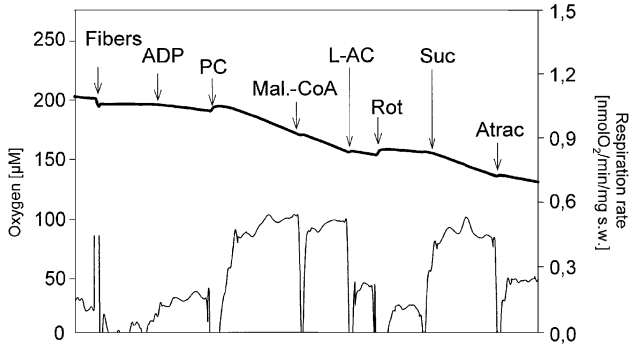

Fig. 6. Respirometric investigation of CPT II activity in human muscle mitochondria. Oxygen consumption of human muscle mitochondria in skinned fibers of m. biceps brachii was recorded using high-resolution respirometry as described previously [21]. Fibers (10 mg) were incubated as described in Section 2.5. Additions: ADP, 2 mM ADP; PC, 300 µM l-palmitoyl-carnitine; Mal.-CoA, 0.5 mM malonyl-CoA; L-AC, 2 mM L-aminocarnitine; Rot, 20 µM rotenone; Suc, 10 mM succinate; Atrac, 10 mM atractylate. Thick line: oxygen concentration versus time of incubation, thin line: rate of respiration.

Table 1
Multiple substrate inhibitor titration for investigation of functional consequences of CPT II inhibition by L-AC

	JO_2 (nmol O_2/mg/min)	(%)
Palmitolycarnitine	0.32 ± 0.05	100
Malonyl-CoA (0.5 mM)	0.33 ± 0.11	103
L-AC (3.4 mM)	0.24 ± 0.04	75
L-AC (37.8 mM)	0.12 ± 0.02	38
Rotenone	0.08 ± 0.01	25
Succinate	0.45 ± 0.03	141

Mitochondrial respiration was measured in human skinned fibers as described in Fig. 6. Data as mean values of eight different incubations ± S.E.

by succinate. State 4 respiration was measured after inhibition of the AdN-translocator with atractylate.

Table 1 summarized the results from eight different respirometric experiments. Obviously, malonyl-CoA did not inhibit the mitochondrial function whereas, L-AC at increasing concentrations increasingly diminished the rates of respiration. The succinate-dependent respiration (0.45 nmol O_2/mg/min) was the same as measured without both inhibitors, indicating that L-AC has no unspecific effects on muscle mitochondria.

In contrast to enzymatic measurements of CPT II, where the enzyme was sensitively inhibited by L-AC, the CPT II-dependent respiration in muscle mitochondria required higher L-AC concentrations to be affected. The most probable reason is a low affinity of L-AC to the acyl-carnitine translocator, therefore, an elevated extramitochondrial concentration is required for L-AC transport into the matrix space. Since other functional properties of mitochondria are obviously not affected by L-AC, the respirometric protocol shown in Fig. 6 is useful for study of consequences of CPT II defects on the level of mitochondrial function. Chiodi et al. [14] investigated the effect of L-AC on the palmitoyl-carnitine-dependent respiration of rat liver mitochondria and found an $IC_{50} = 5$ µM L-AC, which is clearly lower than concentrations required in the experiments presented in this work. This higher sensitivity of liver mitochondria for L-AC inhibition could be caused by a more effective transport via the acyl-carnitine translocator or by an increased sensitivity of rat liver CPT II to L-AC.

Recently, it has been shown that outer membrane permeability for ADP is influenced by oxidation of acyl-carnitines but not when their oxidation was prevented by the absence of necessary cofactors or blocked with rotenone [27]. L-AC should be an appropriate inhibitor to study this effect which could bring more light into the bioenergetic background of rhabdomyolysis.

4. Conclusions

In conclusion, different behaviours of CPT I and CPT II with respect to inhibitors as malonyl-CoA and L-AC allow the investigation of individual kinetic properties of CPT I

and CPT II in homogenates of human skeletal muscle. Since L-AC also inhibits CPT II within intact mitochondria, we propose this technique as an approach to investigate kinetic and functional consequences of decreased CPT II activity in muscle biopsies.

Acknowledgements

We thank Sigma Tau for the supply with L-AC and the Martin-Luther-University Halle-Wittenberg for a grant given to Kathrin Traufeller (Graduiertenstipendium). This work was supported by a grant 3017A/0088H (Kultusministerium Sachsen Anhalt) and from a DFG grants Ge 664/7-1, SFB 598. We further thank Mrs. S. Scholz for skilful technical assistance.

References

[1] J.D. McGarry, N.F. Brown, The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis, Eur. J. Biochem. 244 (1997) 1–14.
[2] S. DiMauro, P.M. DiMauro, Muscle carnitine palmitoyltransferase deficiency and myoglobinuria, Science 182 (1973) 929–930.
[3] M. Huizing, V. Iacobazzi, L. Ijlst, P. Savelkoul, W. Ruitenbeek, L. van den Heuvel, C. Indiveri, J. Smeitink, F. Trijbels, R. Wanders, F. Palmieri, Cloning of the human carnitine-acyl-carnitine carrier cDNA and identification of the molecular defect in a patient, Am. J. Hum. Genet. 61 (1997) 1239–1245.
[4] I. Tein, F. Demaugre, J.P. Bonnefont, J.M. Saudubray, Normal muscle CPT 1 and CPT 2 activities in hepatic presentation patients with CPT 1 deficiency in fibroblasts, J. Neurol. Sci. 92 (1989) 229–245.
[5] S. Zierz, Carnitine palmitoyltransferase deficiency, in: A.G. Engel, C. Franzini-Armstrong (Eds.), Myology, 2nd ed., McGraw-Hill, New York, 1994, pp. 1577–1586.
[6] F. Demaugre, J.P. Bonnefont, G. Mitchell, N. Nguyen-Hoang, A. Pelet, M. Rimoldi, S. DiDonato, J.M. Saudubray, Hepatic and muscular presentations of carnitine palmitoyl transferase deficiency: two distinct entities, Pediatr. Res. 24 (1988) 308–311.
[7] F. Taroni, E. Verderio, F. Dworzak, P.J. Willems, P. Cavadini, S. DiDonato, Identification of a common mutation in the carnitine palmitoyltransferase II gene in familial recurrent myoglobinuria patients, Nat. Genet. 4 (1993) 314–320.
[8] J.P. Bonnefont, F. Demaugre, C. Prip-Buus, J.M. Saudubray, M. Brivet, N. Abadi, L. Thuillier, Carnitine palmitoyltransferase deficiencies, Mol. Genet. Metab. 68 (1999) 424–440.
[9] M. Deschauer, T. Wieser, R. Schröder, S. Zierz, A novel nonsense mutation (515del4) in muscle carnitine palmitoyltransferase II deficiency, Mol. Genet. Metab. 75 (2002) 181–185.
[10] M. Deschauer, Z. Chrzanowska-Lightowlers, E. Biekmann, R.W. Taylor, D.M. Turnbull, S. Zierz, Mol. Gen. Metab. In press.
[11] T. Wieser, M. Deschauer, S. Zierz, Genetics of carnitine palmitoyltransferase II deficiencies, Adv. Exp. Med. Biol. 46 (1999) 339–345.
[12] B.C. Weis, A.T. Cowan, N. Brown, D.W. Foster, J.D. McGarry, Use of a selective inhibitor of liver carnitine palmitoyltransferase I (CPT I) allows quantification of its contribution to total CPT I activity in rat heart. Evidence that the dominant cardiac CPT I isoform is identical to the skeletal muscle enzyme, J. Biol. Chem. 269 (1994) 26443–26448.
[13] S.V. Pande, T.S. Lee, M.S. Murthy, Freeze–thawing causes masking of membrane-bound outer carnitine palmitoyltransferase activity: implications for studies on carnitine palmitoyltransferases deficiency, Biochim. Biophys. Acta 1044 (1990) 262–268.
[14] P. Chiodi, F. Maccari, M.T. Ramacci, Tissue lipid accumulation by L-aminocarnitine, an inhibitor of carnitine-palmitoyltransferase-2. Studies in intact rats and isolated mitochondria, Biochim. Biophys. Acta 1127 (1992) 81–86.
[15] W.C. Hulsmann, C.T. Schneijdenberg, A.J. Verkleij, Accumulation and excretion of long-chain acyl-carnitine by rat hearts; studies with aminocarnitine, Biochim. Biophys. Acta 1097 (1991) 263–269.
[16] D.J. Jenkins, O.W. Griffith, DL-Aminocarnitine and acetyl-DL-aminocarnitine. Potent inhibitors of carnitine acyl-transferases and hepatic triglyceride catabolism, J. Biol. Chem. 260 (1985) 14748–14755.
[17] D.L. Jenkins, O.W. Griffith, DL-Aminocarnitine and acetyl-DL-aminocarnitine. Potent inhibitors of carnitine acyl-transferases and hepatic triglyceride catabolism, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 290–294.
[18] F.N. Gellerich, M. Deschauer, Y. Chen, T. Müller, S. Zierz, Functional impairment of mitochondria in skinned fibers of CPEO patients with single and multiple deletions of mt-DNA correlate with heteroplasmy, Biochim. Biophys. Acta 1556 (2002) 41–52.
[19] A. Kraft, O.V. Wersebe, S. Neudecker, W. Hein, M. Haunschild, D. Skladal, W. Sperl, E. Gnaiger, R. Margreiter, S. Zierz, F.N. Gellerich, Long-term stability of mitochondrial function in human skeletal muscle fibers during cold storage, J. Mol. Med. 739 (1995) B50.
[20] T. Haller, M. Ortner, E. Gnaiger, A respirometer for investigating oxidative cell metabolism: toward optimization of respiratory studies, Anal. Biochem. 218 (1994) 338–342.
[21] S. Zierz, A.G. Engel, Regulatory properties of a mutant carnitine palmitoyltransferase in human skeletal muscle, Eur. J. Biochem. 149 (1985) 207–214.
[22] S. Zierz, R.R. Mundegar, F. Jerusalem, Biochemical evidence for heterozygosity in muscular carnitine palmitoyltransferase deficiency, Clin. Investig. 7 (1993) 77–83.
[23] S. Zierz, A.G. Engel, Are there two forms of carnitine palmitoyltransferase in muscle? Neurology 37 (1987) 1785–1790.
[24] K.J. Wiechelman, R.D. Braun, J.D. Fitzpatrick, Investigation of the bicinchoninic acid protein assay: identification of the groups responsible for color formation, Anal. Biochem. 175 (1988) 231–237.
[25] F.N. Gellerich, W.S. Kunz, R. Bohnensack, Estimation of flux control coefficients from inhibitor titrations by non-linear regression, FEBS Lett. 274 (1990) 167–170.
[26] K.F. Woeltje, M. Kuwajima, D.W. Foster, J.D. McGarry, Characterization of the mitochondrial carnitine palmitoyltransferase enzyme system: II. Use of detergents and antibodies, J. Biol. Chem. 262 (1987) 9822–9827.
[27] A. Toleikis, J. Liobikas, S. Trumbeckaite, D. Majiene, Relevance of fatty acid oxidation in regulation of the outer mitochondrial membrane permeability for ADP, FEBS Lett. 509 (2001) 245–249.